



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Emergence of human angiohematopoietic cells in normal development and from cultured embryonic stem cells

Citation for published version:

Zambidis, ET, Sinka, L, Tavian, M, Jokubaitis, V, Park, TS, Simmons, P & Peault, B 2007, Emergence of human angiohematopoietic cells in normal development and from cultured embryonic stem cells. in L Kanz, KC Weisel, JE Dick & WE Fibbe (eds), *Hematopoietic Stem Cells VI: The Annals of the New York Academy of Sciences*. vol. 1106, Wiley-Blackwell, OXFORD, pp. 223-232, 6th Biennial International Symposium and Workshop on Hematopoietic Stem Cells, Tübingen, 14/09/06. <https://doi.org/10.1196/annals.1392.010>

Digital Object Identifier (DOI):

[10.1196/annals.1392.010](https://doi.org/10.1196/annals.1392.010)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Hematopoietic Stem Cells VI

Publisher Rights Statement:

© 2007 New York Academy of Sciences.

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Published in final edited form as:

Ann N Y Acad Sci. 2007 June ; 1106: 223–232. doi:10.1196/annals.1392.010.

Emergence of Human Angiohematopoietic Cells in Normal Development and from Cultured Embryonic Stem Cells

Elias T. Zambidis^a, Lidia Sinka^b, Manuela Tavian^b, J. V. Jokubaitis^c, Tea Soon Park^d, Paul Simmons^e, and Bruno Péault^d

^aKimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland 21231, USA

^bINSERM U602, Hôpital Paul Brousse, Villejuif 94807, France

^cAustralian Stem Cell Centre, Clayton, Victoria 3800, Australia

^dStem Cell Research Center at Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania 15213, USA

^eThe University of Texas Health Science Center, Houston, Texas 77030, USA

Abstract

Human hematopoiesis proceeds transiently in the extraembryonic yolk sac and embryonic, then fetal liver before being stabilized in the bone marrow during the third month of gestation. In addition to this classic developmental sequence, we have previously shown that the aorta-gonad-mesonephros (AGM) embryonic territory produces stem cells for definitive hematopoiesis from 27 to 40 days of human development, through an intermediate *blood-forming endothelium* stage. These studies have relied on the use of traditional markers of human hematopoietic and endothelial cells. In addition, we have recently identified and characterized a novel surface molecule, BB9, which typifies the earliest founders of the human angiohematopoietic system. BB9, which was initially identified with a monoclonal antibody raised to Stro-1⁺ bone marrow stromal cells, recognizes in the adult the most primitive Thy-1⁺ CD133⁺ Lin[−], non-obese diabetic—severe combined immunodeficiency disease (NOD–SCID) mouse engrafting hematopoietic stem cells (HSCs). In the 3- to 4-week embryo, BB9 expression typifies a subset of splanchnopleural mesodermal cells that migrate dorsally and colonize the ventral aspect of the aorta where they establish a population of hemogenic endothelial cells. We have indeed confirmed that hematopoietic potential in the human embryo, as assessed by long-term culture-initiating cell (LTC-IC) and SCID mouse reconstituting cell (SRC) activities, is confined to BB9-expressing cells. We have further validated these results in the model of human embryonic stem cells (hESCs) in which we have modeled, through the development of hematopoietic embryoid bodies (EBs), primitive and definitive hematopoieses. In this setting, we have documented the emergence of BB9⁺ hemangioblast-like clonogenic angiohematopoietic progenitors that currently represent the earliest known founders of the human vascular and blood systems.

Keywords

hematopoietic stem cell; embryonic stem cell; hemangioblast; embryo; blood vessel

INTRODUCTION

Emerging hematopoiesis adapts to the fast-changing anatomy of the mammalian embryo, proceeding transiently in the extraembryonic yolk sac, then in the liver before stabilizing in the fetal thymus and bone marrow. In the yolk sac, hematopoietic stem cells (HSCs) and endothelial cells emerge simultaneously from extraembryonic mesoderm, leading to joint formation of blood and blood vessels from a putative common stem cell, the *hemangioblast*.^{1,2} It was much later demonstrated that a second phase of *de novo* HSC production takes place within the aorta-gonad-mesonephros (AGM) region of the embryo proper (reviewed in Refs. 3,4). HSCs arising in the AGM sprout from the ventral aspect of the dorsal aorta and vitelline artery, where discrete subsets of vascular endothelial cells exhibit transient blood-forming activity.^{5–7} Besides animal models, this developmental sequence has been also closely scrutinized in the human embryo and fetus, in which the abundance of cell lineage markers and robust functional assays have allowed to precisely document incipient angiohematopoiesis from the third week of gestation.^{8–16}

Pluripotent mouse embryonic stem cells (mESCs) form cellular clusters termed *embryoid bodies* (EBs) that can also differentiate into hematopoietic and endothelial progenitors and recapitulate embryonic hematopoiesis through a hemangioblast intermediate, even producing yolk sac-like blood islands.^{17–19} A clonogenic *hemangioblast* was first characterized as a bipotential yolk sac-type progenitor termed the *blast colony-forming cell* (BL-CFC) derived from mESC²⁰ and mouse embryos.²¹ These VEGF-responsive progenitors arise in yolk sac blood islands and mouse EBs as flk-1/KDR⁺ (VEGF-R2⁺) mesoderm cells.²² The derivation of multipotent human hemangioblasts from human embryonic stem cells (hESCs) has not yet been reported, and remains a sought-after goal, which should allow the unlimited supply of human hematopoietic and vascular cells for transplantation. Since human embryonic and fetal tissues are usually difficult to procure, the basic cellular and genetic mechanisms of human angiohematopoietic cell lineage incipience and development could be investigated using hESC models. We herein report our most recent attempts to understand human developmental hematopoiesis through the parallel exploration of normal embryos and cultured embryonic stem cells. We have notably taken advantage of the identification by some of us of BB9, a novel cell-surface antigen that typifies the most primitive HSCs at adult stages, and a population of candidate hemangioblasts in the emergent human angiohematopoietic system.

BB9, a Novel Marker of Human Early HSCs

In human adult bone marrow, the BB9 antibody exhibits reactivity with a subpopulation of CD34⁺ cells with the phenotypic characteristics of HSCs, namely low to undetectable levels of CD38, and coexpression of CD90 and CD133.²³ Similarly, BB9 expression is highest on CD34⁺CD133⁺CD90⁺CD38[−] cells in fetal liver and umbilical cord blood (UCB). The most direct evidence for BB9 expression by HSCs comes from non-obese diabetic—severe combined immunodeficiency disease (NOD–SCID) mouse transplant studies where CD34⁺BB9⁺ but not CD34⁺BB9[−] UCB cells successfully engrafted and sustained long-term multilineage haemopoiesis in this most rigorous surrogate assay for human HSC activity (Jokubaitis *et al.*; *Pers. Observ.*)

Development of Hematopoiesis in the Human Embryo, from the Splanchnopleura to the Liver Rudiment

As in all other mammals, human hematopoiesis begins in the yolk sac before proceeding to intraembryonic blood-forming organs, upon colonization of the rudiments of the latter by blood-borne HSCs. This pathway set the basis for the prevailing idea that the YS was also the unique provider of HSC, and accordingly no intrinsic hematopoietic potential was

observed in any other tissue of the embryo. Evidence for an intraembryonic emergence of hematopoietic progenitors was obtained in birds, mice, and from our own work conducted in the human embryo. In the mouse, the AGM region, and its anlage, the paraaortic splanchnopleura (P-Sp), are endowed with hematopoietic potential.⁴ Indeed, cells expressing markers of hematopoietic progenitors are present in the dorsal aorta.³ In the human embryo, some of us have identified a dense population of CD34⁺ blood cells adhering to the ventral side of the aortic endothelium,^{15,24,25} which display a cell-surface and molecular phenotype typifying primitive hematopoietic progenitors (CD45⁺, CD34⁺, CD31⁺, CD38⁻, negative for lineage markers, GATA-2⁺, GATA-3⁺, c-myb⁺, SCL/TAL1⁺, c-kit⁺, flk-1/KDR⁺).^{8,10,11} Hematopoietic cell clusters appear precisely in the embryo between the 27th and 40th days of development, only 2–3 days before hepatic colonization by CD34⁺ progenitors.¹¹ In order to define the origin of these aortic progenitors we have set up a miniaturized *in vitro* system for culturing tissue rudiments dissected from human embryos. In that setting, we detected the existence of a blood-forming potential intrinsic to the human intraaortic splanchnopleura as early as day 19 of development, that is, 2 days before the onset of blood circulation. We further showed that the yolk sac only generates progenitors with limited, myeloid developmental ability, whereas precursors emerging autonomously in the presumptive aortic territory are endowed with multilineage lymphomyeloid potential.¹³ These results established that the development of the human blood system is characterized by two independent waves of HSC emergence. The first one occurs in the yolk sac and generates ephemeral progenitors with restricted developmental potential. The second one takes place in the intraembryonic splanchnopleura and gives rise to multipotent HSCs; these intraembryonic progenitors are responsible for the definitive colonization of the liver and therefore are at the origin of all blood cells in the fetus and adult (reviewed in Refs. 15,24,25).

In order to track the earliest forerunners of this intraembryonic bloodforming activity, we have used a novel monoclonal antibody, BB9, which typifies the most primitive human HSCs at adult stages²³ (see also paragraph above). We show that during human development, BB9 is expressed in all blood-forming tissues: P-Sp, yolk sac, fetal liver, and bone marrow. Starting from day 19 of gestation, BB9 is present on cells in yolk sac blood islands. From this stage until 26 days of gestation, BB9 expression identifies rare CD34⁻CD45⁻ cells, in the embryo proper, concentrated in the hemogenic portion of the P-Sp. From the 27th day of gestation, when hematopoiesis occurs inside the embryo, BB9 stains precisely the HSCs that emerge aggregated on the ventral side of the dorsal aorta and surrounding endothelial cells. This pattern is consistent with the hypothesis of a BB9⁺CD34⁻CD45⁻ hemangioblastic precursor migrating from the P-Sp toward the ventral aorta, to give rise to BB9⁺CD34⁺CD45⁺ hematopoietic progenitors and surrounding BB9⁺CD34⁺CD45⁻ endothelial cells. Along this line, preliminary results obtained by culturing cells sorted from 24- to 26-day human embryos indicate that BB9⁺, but not BB9⁻ cells, are endowed with hematopoietic ability. Furthermore, functional hematopoiesis assays performed on fetal liver and bone marrow cells have highlighted a direct relationship between expression of BB9 and hematopoietic incipience in these organs, too. Recent functional analyses carried out both *in vitro* and *in vivo* on purified BB9⁺ cells are in agreement with these observations. Indeed, in fetal liver and bone marrow, long-term culture-initiating cell (LTC-IC) and SCID mouse reconstituting cell (SRC) abilities are restricted to BB9-expressing cells. Altogether, these data define BB9 as a novel marker of very primitive human mesodermal precursors endowed with hemogenic and, possibly, angiogenic potentialities and persists at the surface of human HSCs at embryonic, fetal, and adult stages (Sinka *et al.*, manuscript in preparation).

Hematopoiesis Ontogeny in the Model of hESCs

As mentioned in the paragraphs above, HSCs first emerge during the early weeks of human gestation within the extraembryonic yolk sac, and secondarily within the truncal arteries of the embryo in the AGM region. This second hematopoietic cell wave gives rise to definitive, adult-type HSCs. In both YS blood islands and embryonic aorta, HSCs develop within immediate vicinity of vascular endothelial cells, which secondarily arise from rare mesodermal hemangioblasts. These developmental processes have been elusive, in part because reliable markers of early human hematoenditheliogenesis were lacking. As described in the first paragraph above, we have recently identified a novel marker of undifferentiated HSCs using a new monoclonal antibody, BB9.²³ The pattern of human embryonic BB9 expression is consistent with the hypothesis that CD34⁺ hemangioblasts emigrate dorsally from the p-Sp, and subsequently colonize the ventral aorta to give rise to CD34⁺ hemogenic endothelial cells. Thus, mesodermal BB9⁺CD34⁺ cells may mark the elusive human hemangioblast in the developing human YS and AGM. The rarity of human embryonic tissue makes the detailed analysis of these BB9⁺ AGM/YS progenitors challenging, although such studies are currently in progress in our laboratories. Differentiated hESCs may provide an alternative, more abundant supply of these progenitors for detailed biologic study.

Differentiation of mESCs^{17,18} and hESCs^{26–29} in suspension culture results in the spontaneous formation of cellular clusters termed *EBs*, containing ectoderm, endoderm, and mesoderm (including hematopoietic progenitors).^{30–33} We and others^{33–36} have demonstrated hematopoietic differentiation from hESCs. Based on the sequential expression of hematoendothelial genes, surface markers, and emergence of primitive and definitive CFC, our group first demonstrated that the human embryoid body (hEB) differentiation system mimics the early phases of human yolk sac blood development with mesodermal-hemangioblast differentiation, followed by primitive/definitive erythromyeloid hematopoiesis.^{16,36} Despite the potential for generating adulttype, transplantable HSCs from hESCs, this goal has thus far been elusive. Our group has suggested that this failure is due to the fact that hESCs produce blood similar to that found in the human yolk sac, and is therefore too immature to provide adult-type hematopoietic reconstitution. A detailed developmental biologic approach will be necessary for understanding how to further mature these yolk sac-like progenitors into adult-type transplantable HSCs.

Our main hypothesis is that mesoderm commitment to hemangioblasts occurs during this hEB differentiation. These hemangioblasts represent common progenitors for human primitive and definitive hematopoiesis, and also vascular endothelium. We have recently improved our original methods³⁶ for differentiating hEBs into hematopoietic progenitors using culture conditions supplemented with the mesodermal morphogens BMP4 and VEGF (Zambidis *et al.*, manuscript in preparation). Kinetic analysis of differentiating hEB cells generates hematopoietic progenitors representing primitive and definitive hematopoiesis over a 4-week time course. CFC assays with hematopoietic growth factors (SCF, IL-3, IL-6, GM-CSF, G-CSF, erythropoietin) of day 7–12 hEB cells generate organized, mesodermal-hematoendothelial (MHE) colonies, and a robust wave of primitive and definitive erythropoiesis correlated with increased expression of *SCL/TAL1*, *GATA1*, *GATA2*, *EKLF*, *PU.1*, and *CDX4*. Increases in mRNA levels of these transcription factors coincided with a similarly increasing expression of the hematoendothelial cell markers *CD31*, *CD34*, and *KDR/flk-1*.

Interestingly, MHE clusters bear striking resemblance to classic histologic descriptions of normal intravascular human yolk sac.³⁷ Erythroid progenitors arose in two waves: a primitive wave characterized by CFU with scant adult hemoglobin (HbA) but abundant embryonic and fetal hemoglobins (HbE, HbF), and a definitive wave characterized by CFU

expressing increased amounts of HbA, and decreased amounts of embryonic and fetal globins.

Expression levels for hematopoietic genes and markers peaked at days 6–10 of hEB differentiation, suggesting that a putative coordinated developmental watershed event in hEB hematoendothelial commitment occurs during this time, and predicts the genesis of a hemangioblast at this stage. This hypothesis is corroborated by the burst of *CDX4* expression accompanying *SCL/TAL1* peak expression at day 9 of hEB development, which in turn coincided with the peak of primitive erythroid CFC and subsequent emergence of definitive CFC. Using novel serum-free methodologies, we revealed this progenitor after developing an *in vitro* hemangioblastic blast-colony-forming cell (BL-CFC) assay that detects single clonogenic hEB cells, which could secondarily give rise to adherent endothelial, or primitive and definitive hematopoietic progenitors. Under either serum-free or OP9 bone marrow stromal co-cultures, we demonstrated that BL-CFC colonies contain a common progenitor for both primitive and definitive erythromyelopoiesis and also adherent endothelial cells. Hemangioblastic BL-CFC progenitor formation is correlated directly with the onset of BB9 hEB expression, but prior to the onset of CD34 expression. FACS analysis of isolated, pooled BL-CFC colonies revealed that they are constituted of BB9⁺CD34^{−/lo}CD45[−] hEB cells.

To further test the definitive hematopoietic potential of FACS-purified BB9⁺CD34⁺CD45[−] or BB9⁺CD43⁺CD45[−] hEB cells, we co-cultured sorted cells on a fetal liver stromal line in the presence of early-acting hematopoietic growth factors (Flt3L, TPO, SCF, IL-6). Sorted hEB cells quickly expanded into mononuclear, cobblestone-forming blasts under these conditions and produced a CD34⁺CD45⁺CD38[−] phenotype. CFC analysis of these cells revealed robust GEMM-CFC, GM-CFC, G-CFC, and M-CFC potentials that were indistinguishable from those of CD34-positive-enriched cord blood CFC controls. BB9⁺CD34⁺ hEB cells also gave rise to definitive-type CD56⁺ putative NK cells and CD19⁺ B lymphocytes on OP9 stromal layers. These data collectively suggest that BB9⁺ hEB cells contain the elusive common hemangioblastic progenitor for endothelium, primitive yolk sac hematopoiesis, and most importantly, can further mature into definitive AGM-type CD34⁺CD45⁺CD38[−] progenitors after exposure to a stromal microenvironmental niche.

CONCLUSION

Few concepts in developmental biology are at the same time as ancient and as minimally documented, experimentally, than that of an angiohematopoietic stem cell, or hemangioblast. Whereas hemangioblast-like progenitors have been typified with markers among mouse-dissociated embryonic stem cells or extraembryonic tissues, the clonal development of blood islands and surrounding endothelial cells in the yolk sac has been recently disputed, using multiple color tags to trace the progeny of individual cells.³⁸ While it may appear presumptuous to explore on human cells and tissues the existence of a developmental intermediate that has not yet been fully characterized in the convenient mouse model, we have pursued the study of the incipient human angiohematopoietic system on the following grounds: (a) we have access to human embryos from the third week of development; (b) a large panoply of reliable markers of human vascular cells and blood cells is available; (c) human angiohematopoiesis can be tested functionally in diverse culture and *in vivo* systems; and (d) hESCs can provide robust surrogate models of human histogenesis. We have, indeed, faithfully reproduced primitive and definitive human hematopoiesis in embryonic stem cell-derived EBs, and confirmed in this setting that hematopoietic cells emerge from blood-forming endothelium, a conclusion reached previously using progenitor cells sorted from normal human embryos. The relevance of the embryonic stem cell model

to normal development is further illustrated by our most recent studies of the BB9 cell-surface antigen, which at this point appears as a promising candidate marker of human hemangioblasts. This assumption was first based on the anatomic distribution, from the yolk sac and splanchnopleura to the aortic hemogenic endothelium and derived HSCs, and functional properties of BB9-expressing cells. However, we are currently uncovering that BB9 also marks emerging clonogenic angiohematopoietic progenitor cells in hEBs. If confirmed by clonal analyses currently in progress, these experiments could reveal the existence of the first univocal hemangioblast marker.

Acknowledgments

This work was supported in part by grants from the University of Pittsburgh and McGowan Institute for Regenerative Medicine, by grant Avenir from Inserm, and by grant 4814 from the Association pour la Recherche sur le Cancer to M.T.E.Z. is supported by NIH grant K08 HL077595. We thank Rose Perry for her assistance with the preparation of this report.

REFERENCES

1. Sabin FR. Studies on the origin of blood vessels and of red blood corpuscles as seen in the living blastoderm of chicks during the second day of incubation. *Carnegie Inst. Wash. Pub. N°272, Contrib. Embryol.* 1920; 9:214.
2. Murray PDF. The development ‘*in vitro*’ of blood of the early chick embryo. *Proc. Roy. Soc. Lond.* 1932; 11:497–521.
3. Godin I, Cumano A. The hare and the tortoise: an embryonic haematopoietic race. *Nat. Rev. Immunol.* 2002; 2:593–604. [PubMed: 12154378]
4. Dzierzak E. Ontogenic emergence of definitive hematopoietic stem cells. *Curr. Opin. Hematol.* 2003; 10:229. [PubMed: 12690291]
5. Jaffredo T, Gautier R, Eichmann A, Dieterlen-lievre F. Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny. *Development.* 1998; 125:4575–4583. [PubMed: 9778515]
6. Nishikawa SI, Nishikawa S, Kawamoto H, et al. *In vitro* generation of lymphohematopoietic cells from endothelial cells purified from murine embryos. *Immunity.* 1998; 8:761–769. [PubMed: 9655490]
7. North TE, De bruijn MF, Stacy T, et al. Runx1 expression marks long-term repopulating hematopoietic stem cells in the midgestation mouse embryo. *Immunity.* 2002; 16:661–672. [PubMed: 12049718]
8. Tavian M, Coulombel L, Luton D, et al. Aorta-associated CD34 +hematopoietic cells in the early human embryo. *Blood.* 1996; 87:67–72. [PubMed: 8547678]
9. Charbord P, Tavian M, Humeau L, PÉault B. Early ontogeny of the human marrow from long bones: an immunohistochemical study of hematopoiesis and its microenvironment. *Blood.* 1996; 87:4109–4119. [PubMed: 8639768]
10. Labastie MC, CortÉS F, RomÉo PH, et al. Molecular identity of hematopoietic precursor cells emerging in the human embryo. *Blood.* 1998; 92:3624–3635. [PubMed: 9808556]
11. Tavian M, Hallais MF, PÉault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. *Development.* 1999; 126:793–803. [PubMed: 9895326]
12. CortÉS F, Debacker C, PÉault B, Labastie MC. Differential expression of KDR/VEGFR-2 and CD34 during mesoderm development of the early human embryo. *Mech. Dev.* 1999; 83:161–164. [PubMed: 10381576]
13. Tavian M, Robin C, Coulombel L, PÉault B. The human embryo, but not its yolk sac, generates lympho-myeloid stem cells. mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. *Immunity.* 2001; 15:487–495. [PubMed: 11567638]
14. Oberlin E, Tavian M, Blazsek I, PÉault B. Blood-forming potential of vascular endothelium in the human embryo. *Development.* 2002; 129:4147–4157. [PubMed: 12163416]
15. Tavian M, PÉault B. Embryonic development of the human hematopoietic system. *Int. J. Dev. Biol.* 2005; 49:243–250. [PubMed: 15906238]

16. Zambidis ET, Oberlin E, Tavian M, PÉault B. Blood-forming endothelium in human ontogeny: lessons from *in utero* developmental and embryonic stem cell culture. *Trends Cardiovasc Med*. 2006; 16((3)):95–101. [PubMed: 16546690]
17. Wiles MV, Keller G. Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development*. 1991; 111:259–267. [PubMed: 1893864]
18. Keller G, Kennedy M, Papayannopoulou T, Wiles MV. Hematopoietic commitment during embryonic stem cell differentiation in culture. *Mol. Cell. Biol*. 1993; 13:473–486. [PubMed: 8417345]
19. Vittet D, Prandini MH, Berthier R, et al. Embryonic stem cells differentiate *in vitro* to endothelial cells through successive maturation steps. *Blood*. 1996; 88:3424–3431. [PubMed: 8896407]
20. Choi K, Kennedy M, Kazarov A, et al. A common precursor for hematopoietic and endothelial cells. *Development*. 1998; 125:725–732. [PubMed: 9435292]
21. Huber TL, Kouskoff V, Fehling, et al. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature*. 2004; 432:625–630. [PubMed: 15577911]
22. Kabrun N, Buhring HJ, Choi K, et al. Flk-1 expression defines a population of early embryonic hematopoietic precursors. *Development*. 1997; 124:2039–2048. [PubMed: 9169850]
23. Ramshaw HS, Haylock D, Swart B, et al. Monoclonal antibody BB9 raised against bone marrow stromal cells identifies a cell-surface glycoprotein expressed by primitive human hemopoietic progenitors. *Exp. Hematol*. 2001; 29:981–992. [PubMed: 11495704]
24. PÉault B, Tavian M. Hematopoietic stem cell emergence in the human embryo and fetus. *Ann. N. Y. Acad. Sci*. 2003; 996:132–140. [PubMed: 12799291]
25. Tavian M, PÉault B. The changing cellular environments of hematopoiesis in human development *in utero*. *Exp. Hematol*. 2005; 33:1062–1069. [PubMed: 16140155]
26. Thomson JA, Itskovitz-eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–1147. [PubMed: 9804556]
27. Reubinoff BE, Pera MF, Fong CY, et al. Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat. Biotechnol*. 2000; 18:399–404. [PubMed: 10748519]
28. Amit M, Carpenter MK, Inokuma MS, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev. Biol*. 2000; 227:271–278. [PubMed: 11071754]
29. Pera MF, Reubinoff B, Trounson A. Human embryonic stem cells. *J. Cell. Sci*. 2000; 113:5–10. [PubMed: 10591620]
30. Itskovitz-Eldor J, Schuldiner M, Karsenti D, et al. Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers. *Mol. Med*. 2000; 6:88–95. [PubMed: 10859025]
31. Zhang SC, Wernig M, Duncan ID, et al. *In vitro* differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol*. 19:1129–1133. [PubMed: 11731781]
32. Kehat I, Kenyagin-karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J. Clin. Invest*. 2001; 108:407–414. [PubMed: 11489934]
33. Kaufman DS, Thomson JA. Human ES cells-haematopoiesis and transplantation strategies. *J. Anat*. 2002; 200:243–248. [PubMed: 12033728]
34. Kaufman DS, Hanson ET, Lewis RL, et al. Hematopoietic colony-forming cells derived from human embryonic stem cells. *PNAS*. 2001; 98:10716–10721. [PubMed: 11535826]
35. Chadwick K, Wang L, Li L, et al. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood*. 2003; 102:906–915. [PubMed: 12702499]
36. Zambidis ET, PÉault B, Park TS, et al. Hematopoietic differentiation of human embryonic stem cells progresses through sequential hemato-endothelial, primitive, and definitive stages resembling human yolk sac development. *Blood*. 2005; 106:860–870. [PubMed: 15831705]
37. Bloom W, Bartelmez GW. Hematopoiesis in young human embryos. *Am. J. Anat*. 1940; 67:21–53.
38. Ueno H, Weissman IL. Clonal analysis of mouse development reveals a polyclonal origin for yolk sac blood islands. *Dev. Cell*. 2006; 11:519–533. [PubMed: 17011491]